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(54) Title: METHODS FOR TREATING CONDITIONS ASSOCIATED WITH EXCESS NITRIC OXIDE

(57) Abstract

The present invention provides for the use of a selective condition inhibitory agent for the prophylactic and/or therapeutic treatment of conditions associated with excess nitric oxide (NO). The present invention provides methods of using the selective condition inhibitory agent to treat conditions associated with excess NO. The present invention is based, at least in part, on the discovery that selective condition inhibitory agents treat conditions associated with excess NO, e.g., that level of NO that exists in the subject in excess of that amount necessary to maintain health and which is endogenously-derived and/or exogenously-acquired. The present invention provides for the use of selective inhibitory agents, e.g., agents that selectively inhibit the actions and metabolic transformations of excessive amounts of endogenously-derived and/or exogenously-acquired NO, for prophylactic and/or therapeutic treatments of a variety of conditions, e.g., atherogenesis such as restenosis, hyperplasia, inflammation, and neurodegenerative disorders.

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METHODS FOR TREATING CONDITIONS ASSOCIATED WITH EXCESS NITRIC OXIDE

Background of the Invention

Atherosclerosis is a disease which causes thickening and hardening of the arteries, and which is characterized by lesions of raised fibrous plaque formed within the arterial lumen. Atherosclerotic plaque is commonly treated by means of angioplasty through the use of a balloon-tipped catheter. Other devices, such as atherosclerectomy instruments which remove obstructions by scraping or shaving plaque from the arterial wall, are also utilized in the treatment of atherosclerosis. More recently, laser systems have been proposed for performing angioplasty. In laser angioplasty, a catheter carrying a fiber optic wave guide is passed through a blood vessel, positioned near an obstruction, and then activated to decompose the plaque with laser radiation.

At present, over 300,000 angioplasty procedures are performed each year in the United States. Unfortunately, restenosis, or closure of the blood vessel following angioplasty or other percutaneous revascularization procedures, is a common occurrence following all types of such surgery. For example, approximately 30% of arterial segments dilated by means of balloon-tipped catheters develop significant restenosis, with peak incidents occurring within six months of the intervention. Similar restenosis rates accompany laser angioplasty procedures. Additionally, data from experimental and clinical studies have suggested that re-occurrence of stenotic episodes rise as high as 50% within six months after the first intervention (Riessen *et al.*, JACC 23, 1234 (1994)). When restenosis occurs in the patient, further coronary difficulties arise which can eventually result in strokes, arrhythmias, myocardial infarcts, and even death.

Evidence suggests that intimal hyperplasia or proliferation of smooth muscle cells is a major factor in restenosis. Proliferation of smooth muscle cells is very common in patients after angioplasty, whether or not restenosis occurs. Medial smooth muscle cells, a main component of the arterial wall, proliferate in response to any injury to the arterial wall. Thus, it is not surprising that restenosis occurs after percutaneous revascularization procedures, since these procedures typically crack and tear the arterial wall during the procedure.

Presently, efforts to prevent restenosis typically consist of drug therapy and modern angioplasty techniques. Drug therapy is primarily directed toward the control of restenosis using antiplatelet agents, antiproliferative agents, or antimigratory agents. Typically, the goal of drug therapy is to reduce smooth muscle cell proliferation by attacking

the smooth muscle cells directly, or by affecting processes that promote smooth muscle cell proliferation. (Unfortunately, most of the drugs under investigation are unproven, with unknown efficiency and side effects.)

Certain drugs have been found to be more effective than others in reducing the occurrence of restenosis. These drugs help reduce the necessity of repeating the angioplasty procedure. For instance, there are drugs which show a tendency to inhibit smooth muscle cell growth. Because these drugs can have undesirable side effects, it is not desirable that they be injected as a bolus dose into a peripheral vein and merely allowed to be carried by the bloodstream to the site of the stenotic lesion. Heparin is one such known drug for inhibiting clotting which can be delivered by injection as a bolus and which is known to have disadvantages. For instance, some patients, such as ulcer patients or patients with high blood pressure, are contraindicated by the administration of such large amounts of heparin.

Several neurohumoral factors, such as angiotensin II, and growth factors, including platelet-derived growth factor (PDGF) and basic fibroblast growth factor (FGF), have been implicated in the development of vascular restenosis *in vivo*. The high incidents of vascular reocclusion associated with angioplasty has lead to the development of *in vivo* animal models of restenosis in the search for agents to prevent restenosis. Angiotensin II receptor antagonists, angiotensin converting enzyme (ACE) inhibitors, alpha-adrenoreceptor antagonists and growth factor antibodies have generally produced only a modest, e.g., between 10% and 50%, reduction of vascular restenosis in such animal models. Clinical studies with ACE inhibitors which showed only a moderate protective effect in animal models of restenosis, have failed to demonstrate a significant efficacy in the prevention of restenosis in humans.

Conventional angioplasty techniques, such as percutaneous transluminal coronary angioplasty (PTCA), utilize a balloon-tipped catheter during the procedure. The catheter is passed percutaneously through the vascular system to an obstruction site within an artery or vessel and then inflated via an inflation medium to dilate the area of obstruction. In balloon angioplasty, the outward compression of the balloon stresses the vessel walls, often resulting in cracking or tearing of the wall and injury to the smooth muscle cells. Consequently, balloon angioplasty procedures may contribute to the occurrence of restenosis in patients within the first year after the intervention.

Conventional laser angioplasty procedures are also utilized to remove atherosclerotic obstructions. Typical laser angioplasty procedures utilize continuous wave (CW) lasers. Such lasers, while sufficient to oblate an obstruction, can also cause substantial thermal injury to vessel walls adjacent the obstruction.

Furthermore, because of the occurrence of restenosis after PTCA, other mechanical devices have been developed for use in this situation. A stent is a device (usually made of metal) that can be placed in the artery at the site of the stenosis. The use of an intracoronary stent for the treatment of restenosis has decreased the incidence of associated myocardial infarction and emergent coronary bypass surgery. The proposed mechanism of action of intracoronary stents and the treatment of acute closure or restenosis is the pinning of the intimal tears formed in the smooth muscle cells of the artery between the stent and the arterial wall, thereby maintaining vessel patency.

Although stents have been shown to be effective in restoring vessel patency and in decreasing myocardial ischemia, the exposure of the metal prosthetic surfaces to circulating blood initiates platelet and coagulation reactions that frequently result in thrombus formation and acute thrombotic occlusions of the stent. The occurrence of thrombosis at the stent site can be a life threatening emergency that usually results in an emergent coronary angioplasty or emergent coronary bypass surgery.

Notwithstanding the above procedures, restenosis continues to pose a significant health risk to patients undergoing angioplasty procedures, as well as continuing to be a significant factor compromising the effectiveness of angioplasty and drug therapy procedures.

Summary of the Invention

The present invention satisfies a need to prevent restenosis following coronary procedures, such as angioplasty, and treats other conditions associated with the presence within the subject of excess nitric oxide (NO). The invention provides an agent that treats conditions associated with excess NO.

The present invention is based, at least in part, on the discovery that conditions exist which are associated with excess NO, e.g., that level of NO that exists in the subject in excess of that amount necessary to maintain health and which is endogenously-derived and/or exogenously-acquired. The present invention further provides for the use of selective inhibitory agents to treat such conditions. These agents selectively inhibit the actions and metabolic transformations of excessive amounts of endogenously-derived and/or exogenously-acquired NO which have deleterious effects while protecting that part of the NO generating mechanisms that are maintained intact for normal function. The methods of this invention can be prophylactic and/or therapeutic treatments of a variety of conditions, e.g., inflammation, cancer, and restenosis.

The present invention provides methods for using a selective condition inhibitory agent to treat conditions associated with excess NO. The method of the present invention provides advantages in that it allows a physician to adequately treat a variety of conditions that were heretofore difficult to treat or untreatable. The agents employed to treat the conditions associated with the excess NO result in fewer, if any, side effects in the patient being treated. The method of the present invention can be advantageous over conventional procedures since it is easily adaptable to existing treatments and thus can be used in combination therapies. The present invention provides a method for treating a condition associated with excess NO in a subject by administering to the subject an effective amount of a selective inhibitory agent to treat the condition.

The present invention further pertains to compositions for treating conditions associated with excess NO in a subject. Other aspects of the invention include packaged inhibitory agents that include instructions relating to use, dosage and regimen.

Detailed Description

The present invention pertains to a method of treating a condition associated with excess nitric oxide (NO) in a subject. The method involves the administration of an effective amount of a selective condition inhibitory agent, as described below, to the subject such that the condition associated with the excess nitric oxide is treated.

The term "treating" a condition is intended to include preventing, inhibiting, reducing, or delaying the progression of the condition.

The language "condition associated with excess NO" is intended to include acute or chronic degenerative disorders and/or general and/or injury-induced disease states that are at least partially caused by or exacerbated by having excess NO or metabolites associated with excess NO in the subject. The condition can arise from the presence of excess NO in the subject and/or the presence in the subject of deleterious metabolites, e.g., nitrosamines, of NO formed by the various metabolic transformations involving excess nitric oxide. The condition can include, for example, hyperplasia, atherogenesis, carcinogenesis, neurodegenerative disorders, inflammatory disorders, and auto-immune diseases. A subgenus of the present invention as a method for treating conditions associated with excess NO provided that the condition is not carcinogenesis when the selective inhibitory agent is an ascorbic acid derivative.

The term hyperplasia is intended to include the undesirable and unwanted proliferation of cells or tissue. The undesirable growth can include the growth of neoplasms or the growth of benign cells such as in tissue where the growth is inappropriate. Hyperplasia is intended to include intimal hyperplasia, carcinogenesis and artherogenesis. Intimal hyperplasia preferably includes the internal proliferation of cells of an organ or tissue of the subject, and more preferably the proliferation of vascular smooth muscle cells bordering the inner (luminal) lining of the blood vessel. Atherogenesis preferably includes the formation of stenosis and the development of restenosis in particular.

The term carcinogenesis is intended to include all forms of hyperplasia of the tumorous as well as nontumorous type. Examples of the types of cancer covered by the present invention include breast cancer, skin cancer, bone cancer, prostate cancer, liver cancer, lung cancer, brain cancer, cancer of the larynx, gallbladder, pancreas, rectum, parathyroid, thyroid, adrenal, neural tissue, head and neck, colon, stomach, bronchi and kidneys, and leukemia. Specifically, the tumors associated with the foregoing types of cancer that are treated by the selective condition inhibitory agent of the present invention include basal cell carcinoma, squamous cell carcinoma of both ulcerating and papillary type, metastatic skin carcinoma, osteosarcoma, Ewing's sarcoma, veticulum cell sarcoma, myeloma, giant cell tumor, small-cell lung tumor, gallstones, islet cell tumor, primary brain tumor, acute and chronic lymphocytic and granulocytic tumors, hairy-cell tumor, adenoma, hyperplasia, medullary carcinoma, pheochromocytoma, mucosal neuromas, intestinal ganglioneuromas, hyperplastic corneal nerve tumor, marfanoid habitus tumor, Wilm's tumor, seminoma, ovarian tumor, leiomyomater tumor, cervical dysplasia and in situ carcinoma, neuroblastoma, retinoblastoma, soft tissue sarcoma, malignant carcinoid, topical skin lesion, mycosis fungoide, rhabdomyosarcoma, Kaposi's sarcoma, osteogenic and other sarcoma, malignant hypercalcemia, renal cell tumor, polycythermia vera, adenocarcinoma, glioblastoma multiforma, leukemias, lymphomas, malignant melanomas, epidermoid carcinomas, and other carcinomas and sarcomas.

The neurodegenerative disorder that is treated by the present invention is intended to include all idiopathic, genetic or trauma-induced disorders. Examples include Alzheimer's disease, Huntington's disease, Parkinson's disease, epilepsy, olivopontocerebellar atrophy, Parkinsonian dementia, amyotrophic lateral sclerosis (ALS) (e.g., Guam ALS), Down's syndrome, Korsakoff's disease, multi-infarct dementia, and HIV-induced dementia.

The inflammatory disorders of the present invention include those disorders that result in irritation, injury or infection of various tissues and organs of the subject. Generally, inflammatory disorders are most apparent in the joints and related connective

tissue of the subject, and include osteoarthritis, rheumatoid arthritis, tendinitis, bursitis, and like disorders.

The auto-immune disorders of the present invention are intended to include those disorders that inhibit an immune response by the subject, and specifically those disease states that involve an immune response during which nitric oxide-producing cells, e.g., macrophages, are activated. Specific auto-immune disorders covered by the present invention include multiple sclerosis (MS), allergic encephalomyelitis, autoimmune hemolytic anemia, sympathetic ophthalmia, chronic immune thrombocytopenic purpura (ITP), atopic dermatitis, systemic lupus erythematosus (SLE), autoimmune deficiency syndrome, myasthenia gravis (MG), and other related disorders that are typically caused by aberrant T cells.

Other disorders covered by the present invention include stroke, cataracts, sepsis, thyrotoxicosis, osteoporosis, and asthma.

The term "subject" is intended to include mammals having or being susceptible to a condition associated with excess NO. Examples of such subjects include humans, dogs, cats, pigs, cows, horses, rats and mice.

The term "administering" is intended to include all routes of administration which allow the selective condition inhibitory agent to perform its intended function of treating conditions associated with excess NO. Examples of routes of administration which can be used include injection (subcutaneous, intravenous, parenterally, intraperitoneally, intrathecal, etc.), oral, inhalation, and transdermal. The injection can be bolus injections or can be continuous infusion. Additionally, catheters and other mechanical drug delivery devices (such as stents) can be employed to introduce the agent into the subject and/or to introduce the agent to a specific site for treatment. Examples of catheters that can be employed in the present invention to effectuate site-specific delivery of the inhibitory agent include a double-balloon catheter, a porous balloon catheter, a hydro-gel coated balloon catheter, a catheter having a balloon disposed within a porous balloon, and a microporous balloon catheter (see Wilensky *et al.*, TCM 3, 163-170 (1993)). Other catheter designs not listed above and representing variations of these catheters is deemed to be within the purview of one of ordinary skill, and thus can also be utilized in the present invention. The stent can introduce the agent into the subject by incorporating the agent into the coating that is applied to the stent by known techniques, such as by chemical vapor deposition, ion beam implantation, and plasma deposition. Depending on the route of administration, the selective inhibitory agent can be coated with or disposed in a selected material to protect it from natural conditions which may detrimentally affect its ability to perform its intended function.

One such example includes the use of liposomes to encapsulate or incorporate the agent. The agent can further be encapsulated by a biocompatible and/or biodegradable microparticle, e.g., a polymeric matrix, to effectuate a time-controlled release of the bound agent.

The selective inhibitory agent can be administered alone, or in conjunction with other suitable agents or with a pharmaceutically acceptable carrier, or both. The inhibitory agent can be administered to the subject prior to the onset of the condition, during the condition, or after the onset of the condition. The selective inhibitory agent can also be administered as a prodrug which is converted to its active form *in vivo*.

The language "pharmaceutically acceptable carrier" is intended to include substances capable of being coadministered with the selective inhibitory agent, and which allows it to perform its intended function of treating conditions associated with excess nitric oxide. Examples of such carriers include solutions, solvents, dispersion media, delay agents, emulsions and the like. The use of such media for pharmaceutically active substances are well known in the art. Any other conventional carrier suitable for use with the selective condition inhibitory agent also falls within the scope of the present invention.

The language "effective amount" of the selective condition inhibitory agent is that amount necessary or sufficient to treat conditions associated with excess nitric oxide in the subject. The effective amount can vary depending on such factors as the type of condition being treated, the location of the condition, the type of selective inhibitory agent employed, the potency of the agent, the residence time of the agent within the subject, the size of the subject, the severity of the condition, the mode of application of the agent, and the particular site of administration. For example, the choice of the inhibitory agent can affect what constitutes an "effective amount". One of ordinary skill in the art would be able to study the aforementioned factors and make the determination regarding the effective amount of the selective condition inhibitory agent without undue experimentation. The *in vitro* and *in vivo* assays as described in the Examples below or an assay similar thereto (e.g., differing in choice of condition and inhibitory agent) also can be used to determine an "effective amount" of the inhibitory agent. The ordinarily skilled artisan would select an appropriate amount of the agent for use in the aforementioned *in vitro* and *in vivo* assays. For example, the effective amounts used in the foregoing assays result in a reduction in urinary nitrite/nitrate levels to normal levels, or a reduction in the level of exhaled NO to normal levels.

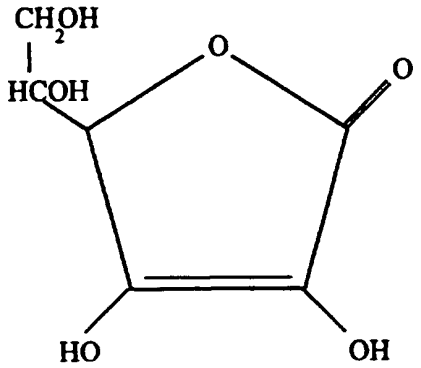
The regimen of administration also can affect what constitutes an effective amount. The selective condition inhibitory agent can be administered to the subject prior to, simultaneously with, or after the onset of the particular condition. Further, several divided

dosages, staggered dosages, as well as a time-controlled release of the agent in selected dosages, can be administered daily or sequentially, or the dose can be continuously infused depending upon the type of agent employed and the preferred mode of administration to the subject. Further, the dosages of the agent can be proportionally increased or decreased as indicated by the exigencies of the therapeutic situation.

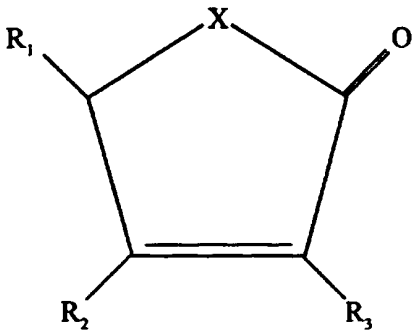
The language "selective condition inhibitory agent" is intended to include agents that selectively inhibit the actions and metabolic transformations of excessive amounts of endogenously-derived and/or exogenously-acquired NO. The inhibitory agent preferably inhibits the deleterious affects of excess NO in the subject by inhibiting selectively the formation of deleterious metabolites involving excessive NO. For example, the agent can inhibit the selected condition by scavenging excess NO in the subject, scavenging nitrite/nitrate, scavenging NO_2, scavenging superoxide anion radicals and/or hydroxyl radicals, and/or "inactivating" the formation or action of metabolites formed in pathways involving NO, such as nitrosamines, preferably by preventing the transformation of the metabolites within the subject into more reactive, e.g., mutagenic or carcinogenic, substances. The selective inhibitory agents are "selective" in that the agents allow for the protection of that part of the NO-generating mechanisms that must be maintained for normal function (via inactivating O_2^-), while eliminating the excess NO that is deleterious, e.g., via inhibiting the formation of or inactivating nitrosamines. The selective inhibitory agents of this invention include anti-oxidants, nitric oxide trappers, nitrite-scavengers, nitrate-scavengers, reductants, and combinations of these agents, as well as other related agents.

The term "anti-oxidants" is intended to include those naturally occurring or artificially synthesized organic compounds that are utilized to retard oxidation. The anti-oxidants of the invention include butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), tert-butylhydroquinone (TBHQ), gallic acid, esters of gallic acid and benzoic acid, vitamins, sulfur compounds, phosphate compounds, caffeic acid, eugenol, ferulic acid, catechol, chlorogenic acid, nordihydroguaiaretic acid (NDGA), flavonoids, e.g., naturally-occurring and synthetic, diketones such as ß-diketones, cysteine such as L-cysteine, glutathione, reduced glutathione peroxidase, tocopherols, analogues and derivatives of ascorbic acid, ellagic acid, hesperidin, hydroquinone, phenylhydrazine, ethoxyquin, esculin, tannic acid, quercetin, myricetin, anthraflavic acid, catalase, mannitol, diallyl sulfide, probucol, superoxide dismutase, ascorbic-6-palmitate, propylparaben, phosphate diesters of vitamins C and E, 2-O-alkylascorbic acids, and 3-O-alkylascorbic acids.

Ascorbic acid, commonly known as vitamin C, and its analogues and derivatives are well known substances (see The Merck Index Eleventh Edition, No. 855 (1989)) and its formula is as follows:



The preferred ascorbic acid derivative of this invention is encompassed by the formula set forth below:



wherein X is selected from the group consisting of N, O, S, NR_4, and CR_5, where R_4 and R_5, if present, are each independently selected from the group consisting of alkyl, alkenyl, alkynyl, and aryl;

R_1 is a moiety selected from the group consisting of hydrogen, hydroxyl, halogen, amino, ester, alkyl, alkenyl, alkynyl, and aryl, haloalkyl, and alkoxyl; and

R_2 and R_3 are each independently a moiety selected from the group consisting of hydrogen, hydroxyl, O-R_6, ester, halogen, alkyl, alkenyl, alkynyl, aryl, haloalkyl, and alkoxyl, wherein R_6 is selected from the group consisting of alkyl, alkenyl, alkynyl, and aryl.

The term "alkyl" refers to the radical of saturated aliphatic groups, including straight-chain alkyl groups, branched-chain alkyl groups, cycloalkyl (alicyclic) groups, alkyl substituted cycloalkyl groups, and cycloalkyl substituted alkyl groups. In preferred

embodiments, a straight chain or branched chain alkyl has 30 or fewer carbon atoms in its backbone (e.g., C_1-C_{30} for straight chain, C_3-C_{30} for branched chain), more preferably 20 or fewer, and most preferably 6 or fewer. Likewise, preferred cycloalkyls have from 4-10 carbon atoms in their ring structure, and more preferably have 5, 6 or 7 carbons in the ring structure.

Moreover, the term alkyl as used throughout the specification and claims is intended to include both "unsubstituted alkyls" and "substituted alkyls", the latter of which refers to alkyl moieties having substituents replacing a hydrogen on one or more carbons of the hydrocarbon backbone. Such substituents can include, for example, a halogen, a hydroxyl, a carbonyl, an alkoxyl, an ester, a phosphoryl, an amine, an amide, an imine, a thiol, a thioether, a thioester, a sulfonyl, an amino, and a nitro moiety. It will be understood by those skilled in the art that the moieties substituted on the hydrocarbon chain can themselves be substituted, if appropriate. For instance, the substituents of a substituted alkyl may include substituted and unsubstituted forms of amines, imines, amides, phosphoryls (including phosphonates and phosphines), sulfonyls (including sulfates and sulfonates), and silyl groups, as well as ethers, thioethers, selenoethers, carbonyls (including ketones, aldehydes, carboxylates, and esters), $-CF_3$, -CN and the like. Cycloalkyls can be further substituted with alkyls, alkenyls, alkoxys, thioalkyls, aminoalkyls, carbonyl-substituted alkyls, CF_3, CN, and the like.

The terms "alkenyl" and "alkynyl" refer to unsaturated aliphatic groups analogous in length and possible substitution to the alkyls described above, but which contain at least one double or triple bond respectively.

Unless the number of carbons is otherwise specified, "lower hydrocarbons" as used herein means an alkyl, alkenyl, and/or an alkynyl group, as defined above, but having from one to three carbons, and more preferably from one to six carbon atoms in its backbone structure. Unless the number of carbons is otherwise specified, "higher hydrocarbons" as used herein means an alkyl, alkenyl, and/or an alkynyl group, as defined above, but having from three to thirty carbons, and more preferably from six to twenty carbon atoms in its backbone structure. Examples of lower hydrocarbon groups which may be used in the present invention include methyl, methylene, ethyl, ethylene, ethenyl, ethenylene, ethynl, ethynylene, propyl, propylene, propenyl, propenylene, propynyl, and propynylene. Examples of higher hydrocarbon groups include butyl, t-butyl, butenyl, butenylene, and butynyl, butynylene, nonyl, nonylene, nonenyl, nonenylene, nonynyl, and nonynylene.

The alkoxyl and haloalkyl groups are alkyl or alkylene groups substituted with one or more oxygen or halogen atoms. The alkoxy and haloalkyl groups may be straight or

branched chain and preferably are made up of up to about ten atoms (including carbon, oxygen or halogen), preferably up to about six atoms, and most preferably up to about three atoms. The term halogen is art-recognized and includes chlorine, fluorine, bromine, and iodine. Examples of substituted hydrocarbon groups which are useful within this invention are similar to hydrocarbon groups set forth above except for the incorporation of oxygen(s) or halogen(s) into the groups.

As used herein, the term "nitro" means $-NO_2$; the term "thiol" means -SH; the term "hydroxyl" means -OH; and the term "sulfonyl" means $-SO_2-$.

The term "thioalkyl" refers to an alkyl group, as defined above, having a sulfhydryl or thioether group attached thereto. In preferred embodiments, the "thioether" moiety is represented by one of -S-alkyl, -S-alkenyl, -S-alkynyl, and $-S-(CH_2)_m-R_7$, wherein R_7 represents an aryl, a cycloalkyl, a cycloalkenyl, a heterocycle or a polycycle and m is zero or an integer in the range of 1 to 8.

The term "carbonyl-substituted alkyl" as used herein means an alkyl group, as defined above, having a substituted or unsubstituted carbonyl group attached thereto, and includes aldehydes, ketones, carboxylates and esters.

The terms "alkoxyl" or "alkoxy" as used herein refer to an alkyl group, as defined above, having an oxygen attached thereto. Representative alkoxyl groups include methoxy, ethoxy, propoxy, tert-butoxy and the like. An "ether" is two hydrocarbons linked by an oxygen. Accordingly, the substituent of an alkyl which renders that alkyl an ether is or resembles an alkoxyl, such as can be represented by one of -O-alkyl, -O-alkenyl, -O-alkynyl, $-O-(CH_2)_m-R_7$, where m and R_7 are described above.

The term "sulfonate" as used herein means a sulfonyl group, as defined above, attached to an alkyl or aryl group. The term sulfate, as used herein, means a sulfonyl group, as defined above, attached to a hydroxy or alkoxy group.

Analogous substitutions can be made to alkenyl and alkynyl groups to produce, for example, alkenylamines, alkynylamines, alkenylamides, alkynylamides, alkenylimines, alkynylimines, thioalkenyls, thioalkynyls, carbonyl-substituted alkenyls or alkynyls, alkenoxyls, alkynoxyls, metalloalkenyls and metalloalkynyls.

The term "aryl" as used herein includes 4-, 5-, 6- and 7-membered single-ring aromatic groups which may include from zero to four heteroatoms, for example, benzene, pyrrole, furan, thiophene, imidazole, oxazole, thiazole, triazole, pyrazole, pyridine, pyrazine,

pyridazine and pyrimidine, and the like. Those aryl groups having heteroatoms in the ring structure may also be referred to as "aryl heterocycle". The aromatic ring can be substituted at one or more ring positions with such substituents as described above, as for example, halogens, alkyls, alkenyls, alkynyls, hydroxyl, amino, nitro, thiol amines, imines, amides, phosphonates, phosphines, carbonyls, carboxyls, silyls, ethers, thioethers, sulfonyls, selenoethers, ketones, aldehydes, esters, or $-(CH_2)_m-R_7$, $-CF_3$, -CN, or the like.

The terms "heterocycle" or "heterocyclic group" refer to 4 to 10-membered ring structures, more preferably 5 to 7 membered rings, which ring structures include one to four heteroatoms. Heterocyclic groups include pyrrolidine, oxolane, thiolane, imidazole, oxazole, piperidine, piperazine, morpholine. The heterocyclic ring can be substituted at one or more positions with such substituents as described above, as for example, halogens, alkyls, alkenyls, alkynyls, hydroxyl, amino, nitro, thiol, amines, imines, amides, phosphonates, phosphines, carbonyls, carboxyls, silyls, ethers, thioethers, sulfonyls, selenoethers, ketones, aldehydes, esters, or $-(CH_2)_m-R_7$, $-CF_3$, -CN, or the like.

The terms "polycycle" or "polycyclic group" refer to two or more cyclic rings (e.g., cycloalkyls, cycloalkenyls, cycloalkynyls, aryls and/or heterocycles) in which two or more carbons are common to two adjoining rings, e.g., the rings are "fused rings". Rings that are joined through non-adjacent atoms are termed "bridged" rings. Each of the rings of the polycycle can be substituted with such substituents as described above, as for example, halogens, alkyls, alkenyls, alkynyls, hydroxyl, amino, nitro, thiol, amines, imines, amides, phosphonates, phosphines, carbonyls, carboxyls, silyls, ethers, thioethers, sulfonyls, selenoethers, ketones, aldehydes, esters, or $-(CH_2)_m-R_7$, $-CF_3$, -CN, or the like.

The ascorbic acid derivatives of the present invention are publically available or can be prepared using art-recognized techniques. Examples of such methods and derivatives are described in Hatanaka *et al.* (U.S. Patent No. 5,008,405), issued April 16, 1991, Suzuki et al. (Jpn. J. Cancer Res. 82, 386-389, April 1991), Kato et al. (Journal of Medicinal Chemistry 31, 793, 1988) and Kushida et al. (Carcinogenesis Vol. 13, No. 6, pp 913-915, 1992) the contents of each are herein incorporated by reference.

The term "nitric oxide trappers" is intended to include compounds that trap NO. Examples include hemoproteins such as hemoglobin, derivatives of hemoglobin such as oxyhemoglobin, heme, hemin, hematin, ovoglobulin, ovoconalbumin, cytochrome C, cytochrome P_{450}, polymerized hemoglobin, ovomucin, and lactalbumin.

The term "nitrite/nitrate-scavengers" is intended to include compounds that scavenge nitrite/nitrate. Examples include ascorbic acid derivatives, urea, phthaloyl

dichloride (PDC), benzene, toluene sulfonyl chloride, and aminosulphonic acid, urea and N-methyl nitroanaline.

The term "reductants" is intended to include those compounds that also retard oxidation by either 1) accepting one or more electrons by an atom or ion, 2) removing oxygen from a compound or agent, or 3) adding hydrogen to a compound. The reductant preferably treats the condition associated with excess NO by selectively inhibiting the deleterious metabolites associated with excess nitric oxide. Examples of reductants utilized in the present invention include alcohol, ether, ketones, aldehyde, azo and diazo compounds, sulfides, bisulfides, hydrosulfides, hydroquinones, and hydrogen peroxide.

The inhibitory agent of the present invention can further be selected according to a list of characteristics that exemplify the preferred functionalities of the agent. The agent can thus be selected on the basis of one or more of the following characteristics: 1) the agent is amphiphilic; 2) the agent is a scavenger of nitrogen dioxide; 3) the agent is an inhibitor of nitrosation; 4) the agent is a scavenger of reactive oxy free radicals; 5) the agent is a scavenger of the superoxide anion radical; 6) the agent is an inhibitor of lipid peroxidation; 7) the agent is a scavenger or trapper of NO; 8) the agent is an inhibitor of the metabolic conversion of nitrosamines into more reactive substances; and 9) the agent has an inherent propensity to act on activated cells.

The selective inhibitory agent of the invention can also be selected on the basis of a set of domains. For example, the agent can have a first domain or moiety that is substantially lipophilic and a second domain or moiety that is substantially hydrophilic.

The language "excess NO" is intended to include both exogenously-acquired and endogenously-derived levels of NO that are above, greater than, or in excess of that level of NO in the subject that is created by both the inducible and constitutive enzymatic pathways and which is necessary for the health and maintenance of the subject. The excess NO can arise from the increased synthesis of endogenous NO, for example, by the induction of an NO-synthesizing enzyme, e.g., NO-synthase, by substances released from cells and/or can arise from the accumulation of exogenous nitric oxide from environmental and/or nutritional sources. Examples of cells that produce NO include macrophages, endothelial cells, astrocytes, neurones neutrophils, hepatocytes, and Kupffer cells.

Excess endogenous NO is deleterious to the subject since it reacts with oxygen and then nitrosates secondary amines to produce carcinogenic/mutagenic compounds, such as nitrosamines. Specifically, nitrosation (the addition of a nitroso (NO) group) involves the reaction of most types of amines with nitrosating agents, such as NO and nitrite

(NO_2^-). NO_2^- is formed *in vivo* by bacterial reduction of NO_3^- and/or by selected cells, such as activated endothelial cells and macrophages. The formation of NO_2^- *in vivo* by cells occurs through the enzymatic oxidation of L-arginine to NO, which reacts with oxygen to form nitrogen dioxide (NO_2). NO_2 exists in equilibrium with several potent nitrosating agents, e.g., N_2O_3 and N_2O_4. Ensuing reactions involving these agents yields nitrosamines, which are deleterious metabolites. The endogenous production of nitrosamines can naturally occur in the subject, such as in the oral cavity, urinary bladder, sigmoid colon, and in the vaginal vault.

The present invention further pertains to packaged condition inhibitors containing a selective condition inhibitory agent as described above, packaged with instructions for using the agent as a treatment for conditions associated with excess NO. The instructions would provide selected information such as the appropriate dose of the agent or the appropriate regimen.

The following invention is further illustrated by the following examples, which should not be construed as further limiting. The contents of all references, pending patent applications and published patents, cited throughout this application are hereby expressly incorporated by reference.

EXAMPLES

Example 1 The *In Vitro* Inhibitory Effect of an Anti-Oxidant On PDGF-NO-Donor Mediated Growth of Cultured Smooth Muscle Cells from Human Primary Stenosing and Restenosing Lesions

The anti-oxidant is administered in this example to illustrate the mitogenic or co-mitogenic effect of nitric oxide (NO) and the effect of an anti-oxidant on the enhanced sensitivity of restenosing cells to growth factors. NO acts as an amplification or progression factor in cell growth.

Cell Line

Smooth muscle cells are isolated from human primary stenosing lesions (ps-SMC) and fresh restenosing lesions (re-SMC) after percutaneous transluminal atherectomy of severely stenosed or completely occluded superficial femoral arteries. The cells are isolated and grown in culture, and then subcultured. Cell growth curves and the effects of growth factors are then determined.

Compounds

In this example, the effects of platelet-derived growth factors (PDGF) (1-5 ng/ml) alone, an exogenous NO-donor such as SIN-1 or an endogenous NO-precursor such as L-arginine alone, and IL-1β (0.1 - 30 ng/ml) alone are tested. Subsequently, PDGF + NO-donor or L-arginine, and PDGF + NO-donor or L-arginine + IL-1β are tested. The preventive action of 2-O-octadecylascorbic acid (10^{-5} to 10^{-8} M, since its EC_{50} for inhibiting lipid peroxidation in rat brain homogenates is 4.3×10^{-6} M) (see Kato *et al*, Medical, Biochemical and Chemical Aspects of Free Radicals, 447-480 (1989)), or other candidate anti-oxidants are tested as inhibitors of growth responses. Preferably, IL-1β is used since PDGF acts as a "competence" factor in the cell proliferation that may be induced by IL-1β (Iida *et al*, Proc. Natl. Acad. Sci. 88, 6560-6564 (1991)). IL-1β is also considered to be useful since it is a potent inducer of the inducible form of NO-synthase, and therefore favors excessive NO formation.

The PDGF is administered to show that re-SMC exhibits enhanced proliferation in response to PDGF, whereas pr-SMC does not respond to PDGF at the concentrations tested. Since NO is involved in mediating the growth response to PDGF in re-SMC, the candidate drug, e.g., 2-O-octadecylascorbic acid, inhibits the growth response since it prevents the formation of excess NO. Furthermore, since the NO-donor or L-arginine enhances the growth response to PDGF, NO serves as a progression or amplification factor in the sequence of events that follows arterial injury, leading to restenosis.

Example 2 The *In Vivo* Inhibitory Effect of an Anti-oxidant On A Stenosis

This antioxidant is administered in this example to illustrate the *in vivo* inhibitory effect of anti-oxidants on a stenosis.

Methods and Materials

Domestic swine are used in this example. Group 1 (control) is treated with only standard food (or the drug vehicle). Group 2 receives 0.01% 2-O-octadecylascorbic acid in their food (low dose), and Group 3 receives 0.1% 2-O-octadecylascorbic acid in their food (high dose). Treatment with the anti-oxidant is started 1 to 2 weeks before balloon injury, e.g., which occurs during the angioplasty procedure, and is continued until at least two weeks after balloon injury. Exogenous NO-donors (e.g., nitroglycerin or SIN-1) or L-arginine (an endogenous precursor to enzymatically synthesized NO) are also tested as possible enhancers of restenosis. In this example, the intimal proliferation of smooth muscle cells that occurs in

response to the balloon injury is essentially complete in fourteen (14) days (see Steele *et al.*, Circ. Res. 57, 105-112 (1985)). The animals receive 325 mg aspirin per day to reduce the incidence of acute thrombosis after balloon injury.

The foregoing example involves the infliction of the balloon injury after the animals are sedated with a combination of ketamine (25 mg/kg), acepromazine (1.1 mg/kg) and atropine (0.6 mg/kg), administered in combination by intramuscular injection in the right femoral artery (other details concerning methodology and drugs is described in Schneider *et al.*, Circulation 88, 628-637 (1993)). Coronary injuries of the left anterior descending coronary artery (LAD) and the left circumflex artery (LCx) are achieved by stretching the vessel wall with an oversized angioplasty balloon, which is inflated three times and then withdrawn from the artery.

The injured coronary artery segments of the LAD and LCx are located with the aid of coronary angiograms, then dissected in block from the heart. Serial 4-mm sections are processed and embedded in paraffin, and then cross-sections (e.g., 4 μm thick) are stained with hematoxylin-eosin, Verhoeff-van Gieson, and Masson's trichrome stains. Each specimen is then evaluated for the presence of intimal proliferation, luminal encroachment, medial dissection, and alteration of the internal and external elastic lamina using a grading system. Morphometric analysis is performed on the section of each artery with the largest neointimal lesion using a computerized IBM-based system. Data is expressed as maximal intimal thickness (in mm), intimal area (in mm^2) and residual lumen (defined as luminal to intimal plus luminal ratio), this latter measurement reflecting the change in vessel geometry after injury and repair. Angiographic analysis is performed to determine the degree of vessel stretch before and after balloon injury, the balloon to artery ratio, and the luminal narrowing at the time of follow-up.

Plasma and urinary nitrite/nitrate (NO_2^-/NO_3^-) concentrations are measured before the intervention, during the intervention, and fourteen days after the intervention. For measurements of NO_2^-/NO_3^-, blood samples are taken and the plasma NO_2^- concentration is determined by first reducing the NO_3^- concentration enzymatically using nitrate reductase from Aspergillus species. Briefly, plasma samples are diluted 1:4 or 1:10 with distilled water and incubated with assay buffer (composition, Mm: KH_2PO_4, 50; NADPH, 0.6; FAD, 5; and NO_3^- reductase, 20 mu; pH 7.5) for 1 hour at 37^0 C. A standard curve for NO_3^- is constructed and the resultant NO_2^- concentrations are determined by chemiluminescence (see Rees *et al*, Br. J. Pharmacol. 114, 689-693 (1995)). Urinary NO_2^-/NO_3^- levels are measured by an automated procedure (see Kanno *et al*, Clin. Exp. Pharmacol. Physiol. 19, 619-625 (1992)). Inducible NO synthase activity is determined in various tissues (e.g., macrophages,

spleen, liver, heart, injured and normal coronary arteries) upon sacrifice of the control and the anti-oxidant-treated animals to provide estimates of NO generation.

Exhaled NO is measured before, during, and after balloon injury (at designated time intervals) using a chemiluminescence analyzer sensitive to NO (2 to 4000 p.p.b., by volume), adapted for on-line recording of NO concentration (see Kharitonov *et al.*, Lancet 343, 133-135 (1994)).

Example 3 The *Ex Vivo* Inhibitory Effect of Anti-oxidants On Restenosis

The anti-oxidant is administered in this example to illustrate the *ex vivo* inhibitory effects of anti-oxidants on restenosis.

Methods and Materials

Balloon-injury via angioplasty procedures to rat carotid arteries induced the production of NO (in 6 and 24 hours) by increasing the NO-synthase activity in the blood vessel wall. This effect is enhanced by the administration of IL-1β (30 IU/ml) to the subject. The increased NO-synthase activity is correlated with decreased reactivity of the blood vessel to phenylephrine and with an accumulation of cyclic-GMP. Generally, vascular smooth muscle cells contain a system that generates NO and this system is activated following vascular injury in vivo. IL-1β mediates the induction of NO-synthase in the injured blood vessel wall. Thus, IL-1β , PDGF and excess NO are all involved in mediating the cell proliferative response to injury that eventually leads to restenosis. The anti-oxidant is administered in this example to show that excess NO mediates the formation of restenosis, and that cyclic-GMP (the "NO receptor") mediates the early growth-programming events that eventually lead to restenosis. Importantly, balloon-injured arteries show an increase in sensitivity to IL-1β.

In accordance with the methods described in Joly *et al.*, Circ. Res. 71,331-338 (1992), rats are pretreated with a candidate drug, such as the anti-oxidant 2-O-octadecylascorbic acid at doses of 0.05%, 0.1% or 1.0% in food (see Konishi *et al*, The Second Joint Meeting of the AACR/ICA February 10-14 (1992)), or 1-100 mg/kg p.o., or 0.3-30 mg/kg i.p. (based on pharmacokinetic data for rats given by Terao S., Medical, Biochemical and Chemical Aspects of Free Radicals, 477-480 (1989)), and the measurements of NO synthase activity, cyclic-GMP formation and responses to IL-1β are compared with the control (basal diet or vehicle-treated) animals.

Example 4 **The *In Vitro* Inhibitory Effect of Anti-oxidants On Inflammatory Disorders**

The anti-oxidant is administered in this example to illustrate the capacity of anti-oxidants to treat *in vitro* inflammatory disorders.

Methods and Materials

Nitrosation of secondary amines typically occurs in stimulated macrophages, a major cell type involved in inflammatory reactions and in the process of "accelerated atherogenesis" (e.g., restenosis). Immunostimulated (activated) macrophages are involved in inflammatory reactions and in responses to injury. Miwa *et al,* Carcinogenesis 8, 955-958 (1987) showed that macrophages (cultured cell lines and freshly-isolated macrophages) produced NO_2^- and N-nitrosomorpholine when they were immunostimulated with Escherichia coli lipopolysaccharide (LPS) in the presence of morpholine (a secondary amine). Several other secondary amines are also N-nitrosated. The secondary amines act to "tap out" some intermediate in the pathway from the precursor to the final products, NO_2^- and NO_3^-.

This example assesses the abilities of anti-oxidants to inhibit the *in vitro* formation of nitrosamines which, upon further metabolic transformation, are involved in the formation of mutagenesis, carcinogenesis, atherogenesis and inflammation disorders.

Macrophage cell lines (e.g., J774.1, PU5-1.8, WEHI-3 or RAW264 and freshly isolated macrophages from C3H/He mice) are used. These cells are cultured in Dulbecco's modified Eagle's medium (pH 7.5) and then supplemented with calf serum (10%). The concentrations of supernatant NO_2^- and NO_3^- are measured. The cells (1.5×10^6/ml) are then incubated with LPS (10 μg/ml) and morpholine (15 mM) for 72 hours at 37^o C.

Nitrosamines are extracted from the cells with dichloromethane and the extracts are analyzed by a gas-chromatography-thermal energy analyzer (see Miwa et al, cited supra). Under these conditions, the cells mentioned above produce NO_2^- and N-nitrosomorpholine, with LPS being required for both processes. The candidate anti-oxidant, 2-O-octadecylascorbic acid, is tested by adding it to the culture medium over a concentration range of 10^{-7} to 10^{-4} M. The formation of N-nitrosomorpholine and NO_2^- by the cells is monitored for the incubation periods 0-12 hours, 24-36 hours and 48-60 hours. Other secondary amines (e.g., diethylamine, dibutylamine and methylbenzylamine) are also used as substrates.

The anti-oxidant is administered in this example to show that NO_2^- and NO_3^- synthesis is a general property of "activated" macrophages, and provides evidence that the formation of NO_2^-, NO_3^- and N-nitrosamines are inhibited by 2-O-octadecylascorbic acid. Consequently, the deleterious effects of excess NO, one of which is nitrosamine formation (see Tannenbaum *et al.*, Am. J. Clin. Nutr. 53, 247S-250S (1991)), are inhibited by the candidate anti-oxidant.

Example 5 The *In Vivo* Inhibitory Effect of Anti-oxidants On Inflammatory Disorders

The anti-oxidant is administered in this example to illustrate the capacity of anti-oxidants to treat *in vivo* inflammatory disorders.

Methods and Material

The experimental immunopathy termed adjuvant-induced arthritis, which involves a T-lymphocyte-mediated delayed hypersensitivity reaction (see Cohen I. R., Annu. Rev. Pharmacol. 9, 567-589 (1991)), is widely used as a model for studying the anti-inflammatory/anti-rheumatic properties of compounds. In Ialenti *et al*, Br. J. Pharmacol. 110, 701-706 (1993), it is shown that adjuvant arthritis induced in rats by M. tuberculosis is exacerbated by L-arginine (an NO precursor) and suppressed by N^G-nitro-D-arginine methyl ester (L-NAME; an NO synthase inhibitor). Also, antigen-stimulated proliferation of T-lymphocytes and the generation of NO_2^- and the release of acid phosphatase from macrophages are all enhanced in L-arginine-treated arthritic rats and reduced in L-NAME-treated animals. These results indicate that endogenous NO enhances adjuvant arthritis by interfering with the activation of T-lymphocytes and/or macrophages. The effect of L-arginine of reducing weight gain and of L-NAME of increasing weight gain are correlated with the respective pro- and anti-inflammatory actions of these substances. The anti-inflammatory action of the candidate anti-oxidant, 2-O-octadecylascorbic acid, is thus determined using this example.

Male Lewis rats (160-180 grams) are housed with food and water ad libitum (12h/12h light/dark cycle; ambient temperature $22 \pm 1^{\circ}$ C), and are allowed to acclimatize to these conditions for six to eight days. Test compounds are dissolved in the animals' drinking water, or given with food (for 2-O-octadecylascorbic acid). Two groups are given tap water, one without any treatment for recording normal values of parameters (naive group), the other to serve as control of the adjuvant arthritis. L-arginine (30 mg/ml) is given in the drinking water from Day 7 to Day 35. 2-O-Octadecylascorbic acid (0.05%-1.0% in food) is

administered during the total course of this regimen to the control arthritic animals and to the animals treated with L-arginine.

Four days after initiating treatment, adjuvant arthritis is induced in all rats except the naive group by a single intradermal injection (0.1 ml) into the right foot pad of 0.3 mg heat-killed M. tuberculosis in Freund's incomplete adjuvant. The inflammatory response is evaluated by measuring the volume of the contralateral (non-injected) hind paw (secondary lesion). Paw volume is determined by plethysmometry immediately after immunization and every seven days for a total period of thirty-five days. Weight gain is also measured on these days (reduced weight gain is a feature of adjuvant arthritis).

Three rats from each experimental group are killed at weekly intervals. The spleens of the sacrificed rats are removed and the lymphocytes of each spleen are separately isolated. T-lymphocyte proliferation assays are carried out (in microwell plates) in which the cells (10^5 cells/well) are stimulated with M. tuberculosis (final conc: 5 μg/ml) and incubated for seven days at 37° C in 5% CO_2 humidified air. One μCi of [^{3}H]thymidine (about 47 Ci/mmol) is then added to each well. After six hours of incubation at 37° C, the cultures are harvested on glass fiber strips and the [^{3}H]thymidine incorporation is measured using a beta counter. At the same time as removal of the spleen, peritoneal macrophages are isolated and plated in 24-well culture plates at a concentration of 2.5×10^5 cells/ml and incubated for 24 hours. Aliquots of the medium are then taken for assay of NO_2^- and acid phosphatase activity (see Ialenti *et al.*, cited supra).

The anti-oxidant is administered to show that the candidate anti-oxidant reverses the pro-inflammatory and other effects (i.e., reduced weight gain, enhanced proliferation of T-lymphocytes, increased production of NO_2^- by macrophages, and increased release of acid phosphatase from macrophages) of L-arginine since these changes are caused by the excessive formation of NO. An exogenous NO-donor also acts like L-arginine in exacerbating adjuvant arthritis, and such an effect is opposed by the candidate anti-oxidant.

Example 6 The *In Vivo* Inhibitory Effect of Anti-oxidants On Inflammatory Disorders

The anti-oxidant is administered in this example to illustrate the capacity of anti-oxidants to treat *in vivo* inflammatory disorders.

Methods and Materials

The concentration of exhaled NO is increased in patients with asthma (see Kharitonov *et al*, Lancet 343, 133-135 (1994); and Persson *et al*, Lancet 343, 146-147 (1994)) and bronchiectasis (chronic dilatation of bronchii and bronchioles) (see Kharitonov *et al*, Clin. Sci. 88, 135-139 (1995)). This increase is caused by the increased expression of inducible NO-synthase in the inflamed lower respiratory tract, which is supported by the findings that asthmatic patients who are treated with inhaled steroids have normal levels of exhaled NO (see Kharitonov *et al*, 1994 cited supra). Thus, in asthma, increased NO formation in the airways resulting from inducible NO synthase expression in epithelial cells amplifies the inflammatory response in the airways (see also Barnes *et al.*, Thorax 48, 1034-1043 (1993)). Consequently, the administration of an anti-oxidant/NO_2^--scavenging therapy (e.g., with 2-O-octadecylascorbic acid) is beneficial to asthmatic patients and such an effect is shown by a corresponding decrease in the amount of NO exhaled by these patients.

Example 7 The *In Vitro* Inhibitory Effect of Anti-Oxidants On Carcinogenesis

The anti-oxidant is administered in this example to illustrate that anti-oxidants inhibit *in vitro* the mutagenic effects and responses of excess NO.

Methods and Materials

This example shows that NO and NO-donors (e.g., spermine-NO complex and nitroglycerin) are mutagenic in the Ames test conducted with Salmonella typhimurium strain TA1535 (reversion of histidine dependence of the cells back to prototrophy mainly via C → T transitions in the hisG46 (CCC) target codon, consistent with a cytosine-deamination mechanism) (see Wink *et al*, Science 254, 1001-1003 (1991)). The study of Arroyo *et al*, Mutation Res. 281, 193-202 (1992) shows that exposure to low concentrations of NO, alone or in combination with NO_2, causes significantly enhanced mutation in this test. The observed mutagenicity requires that the bacteria be actively dividing at the time of exposure to NO or NO_2 , indicating that the nitrogen oxides (NO_x), or their reaction products, function as direct-acting mutagens. NO is a more effective mutagen than NO_2 , but the observed requirement for O_2 indicates that limited oxidation of NO (presumably to NO_2) is necessary.

With regard to anti-oxidants, the mutagenic activity of NO is effectively blocked by β-carotene, tocopherols, and the amphiphilic anti-oxidant 2-O-octadecylascorbic acid, whereas BHT, dimethyl-β-carotene and mannitol also block the mutagenic effects of NO_x but are less effective. Ascorbic acid is ineffective (all measured by assessing the

frequency of reversion to histidine prototrophy in Salmonella typhimurium TA1535 as an indicator of reversal of NO^- or NO_2^- induced genetic damage).

These results show that a lipophilic anti-oxidant is required for optimal blocking of the mutagenic response to excess NO. Since NO is small and highly lipophilic, it is readily accessible to cells via simple diffusion, and reacts intracellularly or in the medium with any dissolved oxygen or oxygen-derived radical that are generated during exposure to NO_x, resulting in the formation of NO_2. NO_2 when combined with NO yields a potent nitrosating agent that reacts with secondary amines to yield carcinogenic N-nitrosamines in both the aqueous and lipid phases.

Example 8 The *In Vivo* Inhibitory Effect of Anti-Oxidants On Carcinogenesis

The anti-oxidant is administered in this example to illustrate that anti-oxidants inhibit *in vivo* the mutagenic effects and responses of excess NO.

Methods and Materials

Transplantable subcutaneous tumors, such as C-3 fibrosarcoma or melanoma, are transplanted into a group of ninety (90) rats. The groups are broken up into three separate groups. Group 1 (control) receives normal food, group 2 (low dose) receives a low dose of 2-O-octadecylascorbic acid (CV-3611) in their food, and group 3 (high dose) receives a high dose of CV-3611 in their food. Each group is further divided into two groups, the first group receiving L-arginine and the second group receiving an exogenous NO-donor, such as molsidomine or its active metabolite 3-morpholino-sydnonimine (SIN-1) or nitroglycerin. The NO-donor and L-arginine are administered to the rats to determine if they are possible enhancers of carcinogenesis.

EQUIVALENTS

Those skilled in the art will recognize or be able to ascertain using no more than routine experimentation many equivalents to the specific embodiments and methods described herein. Such equivalents are intended to be encompassed by the scope of the following claims.

CLAIMS

1. A method of treating a condition associated with excess nitric oxide in a subject, comprising

administering to a subject an effective amount of a selective condition inhibitory agent such that the condition associated with the excess nitric oxide is treated.

2. The method of claim 1 wherein the selective condition inhibitory agent is selected from the group consisting of anti-oxidants, nitric oxide trappers, nitrate-scavengers, nitrite-scavengers, and reductants.

3. The method of claim 1 wherein said selective condition inhibitory agent is an anti-oxidant.

4. The method of claim 3 wherein said anti-oxidant is selected from the group consisting of butylated hydroxyanisole, butylated hydroxytoluene, tert-butylhydroquinone, gallic acid, esters of gallic acid, esters of benzoic acid, vitamins, sulfur compounds, phosphate compounds, caffeic acid, eugenol, ferulic acid, catechol, chlorogenic acid, nordihydroguaiaretic acid, flavonoids, diketones, cysteine, glutathione, reduced glutathione peroxidase, tocopherols, ellagic acid, hesperidin, hydroquinone, phenylhydrazine, ethoxyquin, esculin, tannic acid, quercetin, myricetin, anthraflavic acid, catalase, mannitol, diallyl sulfide, probucol, and superoxide dismutase.

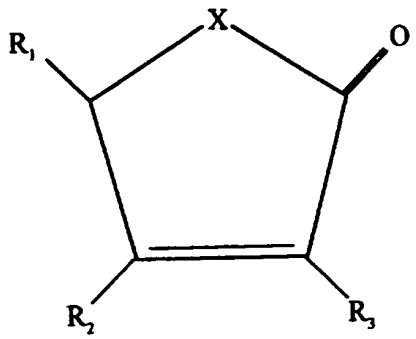
5. The method of claim 3 wherein the anti-oxidant is ascorbic-6-palmitate.

6. The method of claim 3 wherein the anti-oxidant is propylparaben.

7. The method of claim 3 wherein the anti-oxidant is a phosphate diester of vitamins C and E.

8. The method of claim 2 wherein the anti-oxidant comprises derivatives of ascorbic acid.

9. The method of claim 1 wherein the selective condition inhibitory agent is a derivative of ascorbic acid having a formula as follows:



wherein X is selected from the group consisting of N, O, S, NR_4, and CR_5, where R_4 and R_5, if present, are each independently selected from the group consisting of alkyl, alkenyl, alkynyl, and aryl;

R_1 is a moiety selected from the group consisting of hydrogen, hydroxyl, halogen, amino, ester, alkyl, alkenyl, alkynyl, and aryl, haloalkyl, and alkoxyl; and

R_2 and R_3 are each independently a moiety selected from the group consisting of hydrogen, hydroxyl, O-R_6, ester, halogen, alkyl, alkenyl, alkynyl, aryl, haloalkyl, and alkoxyl, wherein R_6 is selected from the group consisting of alkyl, alkenyl, alkynyl, and aryl.

10. The method of claim 3 wherein the anti-oxidant is a diallyl sulfide.

11. The method of claim 3 wherein the anti-oxidant is a 2-O-alkylascorbic acid.

12. The method of claim 3 wherein the anti-oxidant is a 2-O-octadecylascorbic acid.

13. The method of claim 3 wherein the anti-oxidant is a 3-O-alkylascorbic acid.

14. The method of claim 3 wherein the anti-oxidant is a flavonoid.

15. The method of claim 3 wherein the anti-oxidant is a probucol.

16. The method of claim 1 wherein the selective condition inhibitory agent is a nitric oxide trapper.

17. The method of claim 2 wherein the nitric oxide trapper is a hemoprotein.

18. The method of claim 1 wherein the condition is hyperplasia.

19. The method of claim 1 wherein the condition is atherogenesis.

20. The method of claim 1 wherein the condition is restenosis.

21. The method of claim 1 wherein the condition is carcinogenesis.

22. The method of claim 1 wherein the condition is a neurodegenerative disorder.

23. The method of claim 1 wherein the condition is an inflammatory disorder.

24. The method of claim 1 wherein the condition is an auto-immune disorder.

25. The method of claim 1 wherein the selective condition inhibitory agent is administered to the subject intravenously.

26. The method of claim 1 wherein the selective condition inhibitory agent is administered to the subject orally.

27. The method of claim 1 wherein the selective condition inhibitory agent is administered to the subject percutaneously.

28. The method of claim 1 wherein the selective condition inhibitory agent is administered to the subject by a catheter.

29. The method of claim 1 wherein the selective condition inhibitory agent is administered to the subject by a stent.

30. The method of claim 1 wherein the selective condition inhibitory agent is administered to the subject liposomally.

31. The method of claim 1 wherein the selective condition inhibitory agent is administered to the subject prior to the onset of the condition.

32. The method of claim 1 wherein the selective condition inhibitory agent is administered to the subject after the onset of the condition.

33. The method of claim 1 wherein the selective condition inhibitory agent is administered to the subject during the condition.

34. The method of claim 1 wherein the selective condition inhibitory agent is a multi-domain agent having a first domain that is lipophilic and a second domain that is hydrophilic.

35. The method of claim 1 further comprising administering to the subject an effective amount of a second selective condition inhibitory agent.

36. A method for treating a condition associated with excess nitric oxide in a subject, comprising

administering to a subject an effective amount of a compound such that the condition associated with the excess nitric oxide is treated, the compound possessing one or more of the following characteristics,

a) amphiphilicity,
b) capable of being a scavenger of nitrogen dioxide,
c) capable of being an inhibitor of nitrosation,
d) capable of being a scavenger of reactive oxy free radicals,
e) capable of being a scavenger of the superoxide anion radical,
f) capable of being an inhibitor of lipid peroxidation,
g) capable of being a scavenger or trapper of NO,
h) capable of being an inhibitor of the metabolic conversion of nitrosamines into more reactive substances, and
i) an inherent propensity to act on activated cells.

37. A packaged inhibitor for conditions associated with excess nitric oxide, comprising

a selective condition inhibitory agent, packaged with instructions for using an effective amount of the agent for treating a condition associated with excess nitric oxide.

38. A method for treating hyperplasia associated with excess nitric oxide in a subject, comprising

administering to a subject an effective amount of a selective inhibitory agent such that the hyperplasia associated with the excess nitric oxide is treated.

39. The method of claim 38 wherein the selective inhibitory agent is selected from the group consisting of anti-oxidants, nitric oxide trappers, nitrate-scavengers, nitrite-scavengers, and reductants.

40. The method of claim 38 wherein the selective inhibitory agent is an anti-oxidant.

41. The method of claim 40 wherein said anti-oxidant is selected from the group consisting of butylated hydroxyanisole, butylated hydroxytoluene, tert-butylhydroquinone, gallic acid, esters of gallic acid, esters of benzoic acid, vitamins, sulfur compounds, phosphate compounds, caffeic acid, eugenol, ferulic acid, catechol, chlorogenic acid, nordihydroguaiaretic acid, flavonoids, diketones, cysteine, glutathione, reduced glutathione peroxidase, tocopherols, ellagic acid, hesperidin, hydroquinone, phenylhydrazine, ethoxyquin, esculin, tannic acid, quercetin, myricetin, anthraflavic acid, catalase, mannitol, diallyl sulfide, probucol, and superoxide dismutase.

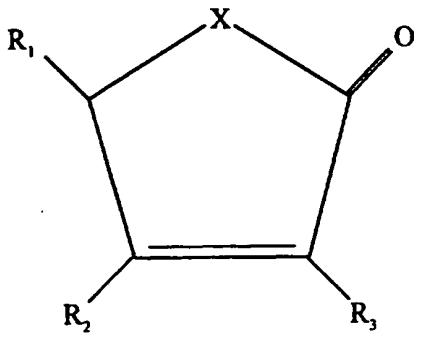
42. The method of claim 40 wherein the anti-oxidant is ascorbic-6-palmitate.

43. The method of claim 40 wherein the anti-oxidant is a propylparaben.

44. The method of claim 40 wherein the anti-oxidant is a phosphate diester of vitamins C and E.

45. The method of claim 40 wherein the anti-oxidant comprises derivatives of ascorbic acid.

46. The method of claim 38 wherein the selective inhibitory agent is a derivative of ascorbic acid having a formula as follows:



wherein X is selected from the group consisting of N, O, S, NR_4, and CR_5, where R_4 and R_5, if present, are each independently selected from the group consisting of alkyl, alkenyl, alkynyl, and aryl;

R_1 is a moiety selected from the group consisting of hydrogen, hydroxyl, halogen, amino, ester, alkyl, alkenyl, alkynyl, and aryl, haloalkyl, and alkoxyl; and

R_2 and R_3 are each independently a moiety selected from the group consisting of hydrogen, hydroxyl, O-R_6, ester, halogen, alkyl, alkenyl, alkynyl, aryl, haloalkyl, and alkoxyl, wherein R_6 is selected from the group consisting of alkyl, alkenyl, alkynyl, and aryl.

47. The method of claim 40 wherein the anti-oxidant is a 2-O-alkylascorbic acid.

48. The method of claim 40 wherein the anti-oxidant is a 2-O-octadecylascorbic acid.

49. A method for treating atherogenesis associated with excess nitric oxide in a subject, comprising

administering to a subject an effective amount of a selective inhibitory agent such that the atherogenesis associated with the excess nitric oxide is treated.

50. A method for treating restenosis associated with excess nitric oxide in a subject, comprising

administering to the subject an effective amount of a selective inhibitory agent such that the restenosis associated with the excess nitric oxide is treated.

51. A method of treating carcinogenesis associated with excess nitric oxide in a subject, comprising

administering to the subject an effective amount of a selective inhibitory agent such that the carcinogenesis associated with the excess nitric oxide is treated.

52. A method of treating neurodegenerative disorders associated with excess nitric oxide in a subject, comprising

administering to the subject an effective amount of a selective inhibitory agent such that the neurodegenerative disorder associated with the excess nitric oxide is treated.

53. A method of treating inflammatory disorders associated with excess nitric oxide in a subject, comprising

administering to a subject an effective amount of a selective inhibitory agent such that the inflammatory disorder associated with the excess nitric oxide is treated.

INTERNATIONAL SEARCH REPORT

Int ional Application No
PCT/US 96/03755

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K31/375

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

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X	WO,A,93 13660 (HEALTH MAINTENANCE PROGRAMS) 22 July 1993 see claims --- -/--	1-4, 18-20, 36-41, 49,50

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

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Date of the actual completion of the international search	Date of mailing of the international search report
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Name and mailing address of the ISA	Authorized officer
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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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A	EP,A,0 609 822 (TAKEDA CHEMICAL INDUSTRIES) 10 August 1994 see page 2, line 8-16; claims -----	1-53

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(information on patent family members)

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PCT/US 96/03755

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